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Efficacy of aminaftone in a rat model of monocrotaline-induced pulmonary hypertension

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ABSTRACT

Pulmonary hypertension is characterized by increased vascular resistances, that could lead to right heart failure and death. Endothelin-1 (ET-1) is a peptide with strong vasoconstrictive and pro-fibrotic properties and is one of the main mediators of pulmonary hypertension. Aminaftone, a synthetic molecule derivative of 4-amynobenzoic acid, down-regulates ET-1 production *in vitro* by interfering with the transcription of the pre-pro-ET-1 gene. The aim of this study was to test whether the inhibition of ET-1 production by aminaftone attenuates the effects of pulmonary hypertension. Pulmonary hypertension was induced through s.c. injection of 60 mg/kg monocrotaline. The rats were randomly assigned to the following experimental groups: Control; Monocrotaline; Aminaftone 30 mg/kg/day; Aminaftone 150 mg/kg/day. After 5 weeks, mortality was significantly lower in the animals treated with aminaftone at both doses compared to monocrotaline alone. Aminaftone reduced plasma concentration of ET-1 and seemed to reduce right heart hypertrophy and the wall thickness of the pulmonary arteries at the highest dose. Aminaftone may represent a novel treatment strategy of pulmonary hypertension.

1. Introduction

Primary pulmonary hypertension is a dreadful clinical condition characterised by sustained elevations of pulmonary artery pressure induced by increased vascular resistances and endothelial remodelling, eventually leading to right heart failure and death (Rubin, 1997). Endothelial disruption and the loss of the natural homeostasis of several cellular pathways and soluble mediators seem to be relevant to the pathogenesis of pulmonary arterial hypertension (PAH). The main mediator of PAH is endothelin-1 (ET-1), since its pulmonary production seems to play a major role in the vascular abnormalities that lead to pulmonary hypertension (Cacoub et al., 1993; Giaid et al., 1993). ET-1 was identified in 1988 (Yanagisawa et al., 1988) as an endothelium-derived peptide with strong vasoconstrictive and pro-fibrotic properties that promote vasospasm, endothelial cell proliferation, smooth muscle hypertrophy in the artery vascular bed (Braun-Moscovici et al., 2004). The development of therapies aimed at decreasing ET-1 detrimental effects on the pulmonary vascular bed via the blockade of ET-1 A and B receptors has dramatically changed the management of pulmonary arterial hypertension, ameliorating the prognosis, the survival and the functional ability of patients (Price and Howard, 2008). Nonetheless, outcome is still bad, not all the patients adequately respond to these therapies and/or the efficacy of treatment may wane during the course of time. Whilst no definitive evidence has been published to explain this individual heterogeneity, it has been postulated that a compensatory rise in ET-1 plasma level following ET-receptor blockade may account for the diminishing efficacy of ET-1 receptor antagonists (Hiramoto et al., 2009). As a consequence, it can be hypothesized that any strategy aimed at reducing ET-1 production may be a valid tool to arrest pulmonary arterial hypertension progression and/or to potentiate the efficacy of ET-1 receptor antagonists in the management of pulmonary arterial hypertension.

We have recently shown that aminaftone (C18-H15-N-O4), a synthetic molecule derived from 4-aminobenzoic acid, down-regulates ET-1 production *in vitro* by interfering with the transcription of the pre-pro-ET-1 gene (Scorza et al., 2008a). In the present study we assessed whether the inhibition of ET-1 production by aminaftone is a valid strategy to prevent the development of pulmonary hypertension after monocrotaline administration, a well-characterised rodent model of pulmonary arterial hypertension (Kay et al., 1967; Roth and Reindel, 1991).

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2. Materials and methods

2.1. Animals

Male Wistar rats weighing 300 ± 30 g were purchased from Charles River Laboratories (Calco, Italy). Procedures involving animals and their care conformed to institutional guidelines in compliance with national (4 D.L. N.116, G.U., supplement 40, 18-2-1992) and international (EEC Council Directive 86/609, OJ L 358, 1, 12-12-1987, National Institutes of Health's Guide for the Care and Use of Laboratory Animals, and US National Research Council 1996) law and policies. All rats were housed in climate-controlled conditions and had free access to chow and water.

2.2. Monocrotaline treatment

Monocrotaline (Sigma) was dissolved in 1 N HCl, and the pH was adjusted to 7.4 with 1 N NaOH. Monocrotaline was administered as a single subcutaneous (s.c.) injection (60 mg/kg) in a volume of 3 ml/kg. Control, age-matched rats received an equal volume of vehicle.

2.3. Experimental design

The animals were randomly assigned to one of the following four experimental groups: 1) control healthy rats ($n=11$); 2) only injection of monocrotaline ($n=12$); 3) injection of monocrotaline followed by aminaftone 30 mg/kg treatment (Aminaftone30) ($n=8$); and 4) injection of monocrotaline followed by aminaftone 150 mg/kg treatment (Aminaftone150) ($n=8$). Aminaftone was administered as food admix. Rats were weighed twice a week.

2.4. Right ventricle pressure measurement

Five weeks after monocrotaline or vehicle injection, all surviving rats were anesthetized with an intraperitoneal injection of 60 mg/kg pentobarbital sodium. A Millar SPR671 catheter was inserted through the right jugular vein and advanced into the right ventricle. The catheter was connected to a pressure-control unit and interfaced with a Gould signal amplifier. Right ventricular systolic pressure was recorded and analysed (Power Lab data acquisition system, AD Instrument). Right ventricular systolic pressure is assumed to be equal to the pulmonary artery systolic pressure in the presence of a normal pulmonary valve (Jones et al., 2002).

2.5. Measurement of organ weight

At the end of the study, the animals were sacrificed by a 2.5 M KCl i.v. injection. The heart was excised, the right ventricle, right atrium and the left ventricle plus septum were separated and weighed; the right ventricular weight/body weight ratio was used as index of right ventricular hypertrophy.

2.6. Measurement of plasma ET-1 levels

At the end of the hemodynamic measurements blood was collected on 5% EDTA from anesthetized rats for ET-1 measurement. Plasma ET-1 concentration was measured using a human ET-1 immunoassay kit (QuantiGlo, QET00; R&D Systems).

2.7. Histological analysis

Right ventricular free wall was fixed by immersion in 4% buffered formalin and embedded in paraffin. 5 μm-thick sections were cut and stained with hematoxylin and eosin. Analysis was done in a blinded fashion. The cross-sectional area (CSA) of cardiomyocytes was determined by manually tracing the cell contour on a digitized image acquired on the image-analysis system at 400× magnification, as already described (Fiordaliso et al., 2007). The CSA of 30–40 cardiomyocytes per heart was then averaged. As an index of media hypertrophy, the external diameter and arterial wall were measured in almost 15 muscular pulmonary arterioles in each rat by the use of Cell F (version 5.0.1175, Olympus) on digitized images at 400× magnification, after hematoxylin and eosin staining. Vessels with a diameter comprised between 40 and 70 μm were analyzed. Relative wall thickness (WT) was calculated as $(WT1+WT2)\times 100$/external diameter, where WT1 was measured at one point of the arterial wall and WT2 at the diametrically opposite point (Beppu et al., 2004).

2.8. Statistical analysis

Data are presented as mean ± standard error of the mean (S.E.M.). Analysis of variance was used to compare all groups, followed by Dunnett's test for multiple comparisons, using untreated Monocrotaline group as reference (GraphPad Prism 5, version 5.03). Survival characteristics of the Aminaftone 30, Aminaftone 150 and the Monocrotaline group were compared by the log-rank test statistic. A $P<0.05$ was chosen as threshold for statistical significance.

3. Results

3.1. Survival

At the end of the experiment, no rats died in the control and Aminaftone 150 groups, while mortality was 38% in the Monocrotaline group and 13% in the Aminaftone 30 group. Overall, aminaftone-treated rats had a significantly lower mortality compared to rats in the Monocrotaline group ($P=0.044$, log-rank test statistic; Fig. 1).

3.2. Body weight

Body weight at the beginning of the experiment was similar in the four groups (control: 327 ± 10 g; Monocrotaline: 309 ± 31 g; Aminaftone 30: 309 ± 33 g; Aminaftone 150: 307 ± 32 g; $P=0.63$). Five weeks after monocrotaline injection, body weight increased more in the control group (+27 ± 2%) than in the Monocrotaline group (+15 ± 7%; $P=0.045$). Rats treated with aminaftone at both doses showed an increase of 20 ± 9% in body weight.

3.3. Cardiac hypertrophy

Right ventricular weight, normalised by body weight, was significantly higher in the Monocrotaline treated rats than in the control group (1.31 ± 0.06 mg/g vs. 0.49 ± 0.03 mg/g, $P<0.0001$).

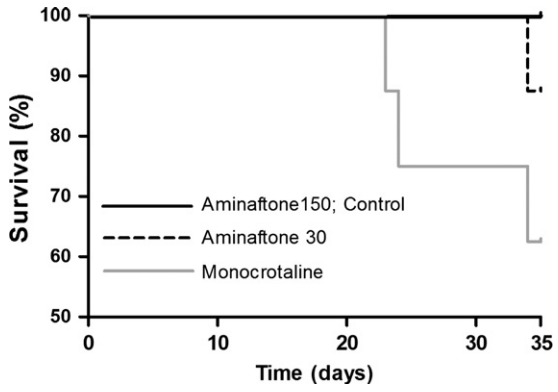

Fig. 1. Kaplan–Meier survival curves for the different experimental groups. Monocrotaline (grey line), Aminaftone 30 (dashed line) Aminaftone 150 (black line); ($P=0.044$ by log-rank test). (control group; black line).